2.—Crinia insignifera Moore (Anura: Leptodactylidae) on Rottnest Island

By A. R. Main

Manuscript received—16th August, 1960

A population of *Crinia insignifera* Moore occurs on Rottnest Island. This species is polymorphic but only two (ridged and lyrate) of the three or possibly four morphs (ridged, lyrate, warty, smooth) known from the mainland occur on the island. It is suggested that the polymorphic characters are a simple Mendelian system and on Rottnest one homozygote is lethal. The frequency of the polymorphs in a local Rottnest population is traced from 1955 to 1960. Morph frequencies remain unchanged until the dry winter in 1959 when a significant change in frequency occurred. The change is possibly related to the seasonal conditions. Estimates of population size were made at the time morph frequencies were scored. These estimates indicate a considerable fluctuation in population size.

The findings are discussed in relation to the depauperate frog fauna found on Rottnest and the possible advantages of the ridged morph under the dry conditions of 1959.

Introduction

The granular-bellied species of *Crinia* (the *signifera* and *insignifera* super-species (Main, Lee and Littlejohn 1958) plus *C. georgiana*) exhibit a considerable variability in dorsal skin pattern. Quite recently some taxonomists (e.g. Loveridge 1935) treated these morphological variants as species. However, Parker (1940) recognised that similar variants occurred in all the granular bellied species and (op. cit. p. 80) associated pattern and skin texture with names as follows: smooth uniform, *affinis;* striped, *stolata or ignita*; and irregularly marbled and warty, "typical" form.

Moore (1954) was the first author who referred to the foregoing phenomenon as polymorphism. Main (1957) distinguished four "morphs" as follows:—"ridged" for *ignita* or *stolata;* "smooth" for *affinis;* "lyrate" for *typical*; "warty" for animals with warty dorsum and lacking ridges. A thorough examination of polymorphism in mainland species is a long term project. The data available so far indicate that the phenotypes ridged and lyrate are genetically distinct but they do not indicate whether warty is also distinct. Furthermore, in some cases it is difficult to distinguish the smooth and warty phenotype. Clearly the basis for distinguishing the phenotypes can only be obtained by breeding several generations and at present this has not proved practicable.

The foregoing applies to mainland populations. There are, however, populations of *Crinia insignifera* Moore on Rottnest Island and, as will be seen below, these offer a simpler situation for a preliminary analysis of such aspects as inheritance, fecundity, viability, yearly changes in morph frequency, and population size.

* Zoology Department, University of Western Australia, Nedlands, Western Australia.

Taxonomic Status of Rottnest Island Populations

Morpologically the island animals are similar to those from the adjacent mainland. Main (1957) has reported a number of crosses between the island and mainland populations of *C. insignifera* which he interpreted as indicating absence of marked genetic incompatibility beween them. A further series of 4 pairs of crosses have been made between Rottnest Island frogs and those from Seaforth and Attadale on the mainland. In each pair of crosses the same female was used, in one cross of the pair the male was from the same locality as the female (control cross) while in the other (experimental cross) it was not. In the control crosses a total of 274 eggs was used and in the experimental 314. There was no significant difference in the number of larvae hatching from the experimental crosses compared with the controls thus the results of these crosses support the earlier conclusions.

Littlejohn (1960) has reported his analysis of male calls and finds no differences. These findings suggest that the island population should be regarded as specifically the same as that of the adjacent mainland.

Nevertheless, despite the absence of behavioural or genetic barriers to gene flow there is one difference between the island and the mainland populations, and this is the absence of "warty" and "smooth" (=patternless) animals from the island population. This observation has remained true for a number of years and has been repeated by many observers.

Inheritance of Polymorphic Characters

In all crosses so far made in which both parents were ridged, the offspring have been ridged. This phenotype must therefore be homozygous for the genetic factors determining dorsal pattern. Furtherfore, ridged cannot be dominant. In Table I, are presented results of two crosses which can be interpreted if it is

TABLE I

Mendelian segregation of phenotypes in F_1 of two *in vitro* crosses of *C. insignifera* from Rottnest Island.

Mating No.	Parents ♀	Parents ♂	Ratio expected	Offspring Ridged	Offspring Lyrate
470	ridged	lyrate	1 : 1	21	18
472	lyrate	lyrate	1 : 2 : 1	6	16
				10 neither ridged nor lyrate died before metamorphosis complete	

assumed that the lyrate phenotype is heterozygous. Mating 470 gives the expected 1 : 1 ratio when the homozygote and heterozygote are mated, while 472 gives the expected 1 : 2 : 1 ($\chi^2 = 1.0$ $p = < .7 > .5$) when both parents are heterozygous. This interpretation assumes that the 10 animals which died at a stage when the ridged or lyrate pattern would have been apparent were neither ridged nor lyrate but patternless and lethal. While the foregoing seems to be an adequate explanation for the Rottnest population, it does not at present offer a satisfactory explanation for mainland populations in which the warty phenotype occurs. When warty animals are used in crosses, some of the offspring are ridged so the warty phenotype is either the lyrate genotype with poor penetrance, or another allele of the ridged locus or an epistatic gene is involved. The selection of the interpretation which satisfactorily accounts for the inheritance among mainland *C. insignifera* requires the rearing and mating of an F_1 generation. Nevertheless, it is possible to take advantage of the apparent genetic simplicity in the Rottnest population and proceed with an analysis of the population assuming that one gene and one allele is involved and that one homozygote is lethal, so that there are only two genotypes and two phenotypes in the population.

Fecundity, Viability and Vigour

Table II presents information collected to date on the number of eggs per morph for Rottnest and Attadale. The number of eggs per morph

TABLE II

C. insignifera, Rottnest and Attadale, mean number of eggs per morph (1956 and 1957 combined).

	Ridged	Lyrate	Patternless
Locality	N. Mean	N. Mean	N. Mean
Rottnest	4 191 ± 31	4 176 ± 45	....
Attadale	8 122 ± 54	15 98 ± 43·5	12 142 ± 51

on Rottnest is not significantly different between ridged and lyrate phenotypes with the small numbers available. Also, the differences between the three phenotypes at Attadale are not statistically significant. There appears to be a considerable difference between Attadale and Rottnest. Differences between these localities were very marked in 1957. However, more data collected during the dry year 1959 tended to raise the mean values for Attadale to ridged 156, lyrate 117. An analyisis of variance shows that, for the Attadale population, differences between morphs in the average number of eggs produced per female are not significant. When all data for each locality are combined the average number of eggs per female is: Attadale 134, Rottnest 183. Analysis of variance shows that this difference is significant at the level $p = <0.05 > 0.01$.

How much biological significance should be attached to this result is uncertain because large (i.e. old) females produce more eggs than young ones which means that in years in which numerous females are breeding for the first time the average number of eggs per female may be small, whereas in years in which few females are breeding for the first time, i.e. there is a high proportion of large old animals (reflecting the poor recruitment resulting from low breeding success in a previous year) the average number of eggs per female may be high.

Crosses carried out and reported earlier (Main 1957) were terminated at the time of hatching of the larvae. More recent breeding experiments (see above) have been continued after hatching. At hatching, the crosses between Rottnest and mainland localities produced slightly smaller offspring than the comparable control. This relationship held in all pairs as the larvae grew except one in which the control cross (lyrate female x ridged male) was much smaller and showed developmental defects such as absence of limb buds, mouth and intestine. None of this cross survived to metamorphosis which suggests that a lethal combination is present in the Rottnest population. However, it is not expressed when eggs of the same female are mated to an Attadale male so a genetic interaction is indicated rather than any abnormality in the eggs. During the 1960 winter this cross has been repeated. At 28 days post hatching no developmental defects as described above are apparent and in length and developmental stages the experimental crosses are comparable with the controls.

The 1960 larvae need to be raised to metamorphosis in order to confirm that they are completely normal but the results suggest that the earlier abnormal larvae were due to an experimental error.

Changes in Morph Frequency

During the dry season (November to April) Crinias are cryptozoic and restricted to the very few sites where there is sufficient cover. In many localities it is impossible to locate any animals despite the fact that very large breeding congresses are formed in these same sites during the breeding season. Fortunately on the north-east side of the Lighthouse Soak (see map in Hodgkin & Sheard (1959)) an area of *sporobolus virginicus* with a large number of small rocks harbours an unknown fraction of the soak population of *C. insignifera* and these have been scored for morph frequency with the results shown in Table III.

In the table sexes have not been distinguished because neither the pre-breeding adults nor the juveniles in the post-breeding populations can be certainly sexed on externals. The data available (unpublished) indicate that, for the Rottnest population, there is no difference in polymorph frequency between the sexes. From 1954 to 1959 the morphs do not differ significantly from equality. However the morph frequencies show a consistent excess of ridged over lyrate animals except in the 1959 pre-breeding population when the two morphs are equally frequent. The post-breeding 1959 population does, however, differ significantly from equality ($p = < 0.02 > 0.01$).

There is no reason to believe that the morph frequencies should be equal and it is more

meaningful to test whether subsequent samples differ significantly from the immediately preceding samples. This has been done with the results shown in the column headed significance in Table III. The changes do not reach statistical significance until post-mating 1959.

TABLE III

Crinia insignifera, Rottnest Island. Frequency of each morph present.

	Frequency		Significance	
	Both sexes			
	Ridged	Lyrate	χ^2	p
1954—				
Rottnest; material added to collection	20	14		
			0·08	< ·8 > ·7
1955—				
Lighthouse Soak (scored in field):				
Pre-breeding	43	34		
			0·262	< ·7 > ·5
Post-breeding	126	114		
1956—				
Lighthouse Soak:			0·931	< ·5 > ·3
Pre-breeding	26	17		
			0·892	< ·5 > ·3
1959—				
Lighthouse Soak:				
Pre-breeding	49	50		
			6·45	< ·02 > ·01
Post-breeding	54	25		
			0·439	·5
1960—				
Lighthouse Soak:				
Pre-breeding	41	24		

The change in morph frequency between pre- and post-breeding 1959 is significant ($p = < 0.02 > 0.01$) and it is possible that these changes are related to the seasonal weather. The 1959 season was warmer and drier than usual, June was the warmest since 1949, July had the highest mean maximum temperature ever recorded and August was the warmest ever recorded. On Rottnest in the period May to September the average rainfall is about 24 inches, but during 1959 it was 7 inches below average.

At Perth the season was also drier than usual but the cloudiness was not much reduced. However, with higher air temperatures and reduced rainfall, water levels in ponds were lower, with a consequent rise in temperature. Minima and maxima were measured on Lighthouse Soak on two occasions, viz., August 15-16 and September 28-29; both minima were 54°F and maxima 71° and 73° respectively. In normal years the maxima are about 10°F lower while the minima are in the vicinity of 50-54°F. In view of the foregoing it seems likely that the change in morph frequency between the pre-breeding and post-breeding population is in some way related to the success of the ridged morph under warmer conditions.

Population Size

Estimates of the size of discrete local populations is possible under two circumstances:

(a) During the dry season when the animals are aggregated in a way that allows sampling (see above).

(b) When water is present and males have formed a breeding congress.

Sampling and estimation are not possible when water is present but conditions are otherwise unsuitable for breeding as the population is then dispersed.

The size of the population on Lighthouse Soak during the dry season has been estimated by toe clipping in a capture, mark, release and recapture procedure. The data are arranged in a manner suggested by Hayne (1949). The formula $P = \Sigma wx^2/\Sigma wxy$ (where P is population estimate) permits an average of the data and yields a population estimate. For the post-breeding population in 1955 the data of Table IV yields a population estimate of 1,021. No further

TABLE IV

Lighthouse Soak, Rottnest—Census data pertaining to the population of *Crinia insignifera*, November, 1955.

Date	Number of captures			Proportion of catch previously handled (y)	Total number previously marked (x)
	New	Previously marked	Total No. (w)		
16/11/55	65	0	65	0·00	0
18/11/55	67	7	74	0·094	65
20/11/55	64	7	71	0·099	132
21/11/55	18	6	24	0·250	196
24/11/55	26	6	32	0·19	214

estimates were possible until 1959 when data obtained from the pre-breeding population (Table V) yields an estimate of 138. The post-breeding population was sampled and the data

TABLE V

Lighthouse soak, Rottnest—Census data pertaining to a population of *Crinia insignifera*, March, 1959.

Date	Number of captures			Proportion of catch previously handled (y)	Total number previously marked (x)
	New	Previously marked	Total No. (w)		
17/3/59	47	0	47	0·00	0
18/3/59	33	15	48	0·31	47
19/3/59	19	28	47	0·60	80

(presented in Table VI) yield a population estimate of 261 animals. The pre-breeding population data for 1960 yields an estimate of 86 animals (Table VII).

TABLE VI

Lighthouse Soak, Rottnest—Census data pertaining to a population of *Crinia insignifera*, November, 1959.

Date	Number of captures			Proportion of catch previously handled (y)	Total number previously marked (x)
	New	Previously marked	Total No. (w)		
18/11/59	6	0	6	0·00	0
21/11/59	10	0	10	0·00	6
22/11/59	40	2	42	0·05	16
23/11/59	21	6	27	0·22	56

TABLE VII

Lighthouse Soak, Rottnest—Census data pertaining to a population of *Crinia insignifera*, March, 1960.

Date	Number of captures			Proportion of catch previously handled (y)	Total number previously marked (x)
	New	Previously marked	Total No. (w)		
8/3/60	32	0	32	0·0	0
9/3/60	18	10	28	0·36	32
10/3/60	15	22	37	0·59	50

In the absence of an estimate of population size based on a breeding congress to check on the above, it would be unwise to consider the foregoing as estimates of the total population because an unknown fraction of the population may be in hiding in undetected places other than stones. Nevertheless, the stones under which the animals are hiding during the dry season have remained constant in number and apparently favourable environmentally because frogs are always found beneath them so it seems reasonable to accept the estimates but interpret them as indicators of the population size over the years.

These estimates indicate a considerable fluctuation in the size of the population on Lighthouse Soak. It is unfortunate that no estimate is available for the pre-breeding 1956 population. Nevertheless it appears that as the population declined from the high numbers of November, 1955 to the low numbers of March, 1959 so the frequency of the ridged morph decreased in the population. The recruitment from breeding in 1959 reversed this trend with the result that at the end of the year ridged animals were twice as abundant as lyrate animals. This change is probably associated with the selective advantage possessed by ridged animals under warmer conditions as discussed in the previous section. In the 1960 pre-breeding populations the excess of ridged animals was maintained (Table III) suggesting that there had not been a strong selection against ridged adults during the period November, 1959-March, 1960. However, during this period deaths had occurred as indicated by the population estimates.

Discussion

Storr, Green and Churchill (1959, p. 70) have shown that the vegetation of Rottnest was once like the tuart woodland of the adjacent mainland but has subsequently changed to a coastal complex. On the mainland from the littoral to the eastern boundary of the tuart woodland ten species of frogs are known to occur. Reasoning from the floral information cited above, all the frogs of the tuart zone could be expected on Rottnest. However, only three, viz. *Crinia insignifera, Hyla moorei* Copeland, and *Heleioporus eyrei* (Gray) occur on the island while seven, viz: *Hyla adelaidensis* Gray, *Limnodynastes dorsalis* (Gray), *Crinia glauerti* Loveridge, *C. georgiana* Tschudi, *Pseudophryne guentheri* Boulenger, *Neobatrachus pelobatoides* (Werner), and *Myobatrachus gouldii* (Gray) do not.

The foregoing would indicate that the faunistic diversity on the island has been reduced in a manner similar to the reduction among plants. Furthermore it seems that the genetic diversity within the *C. insignifera* population has been reduced, at least patternless morphs do not occur on the island where they may now be at a selective disadvantage.

Until the post-breeding sample of 1959 the data suggest that there was a system of balanced polymorphism operating on Rottnest. The increase in frequency of ridged animals during the 1959 breeding season suggests that this genotype has an advantage under the warmer and drier conditions which characterised this season.

The advantage enjoyed by striped morph may be due to

(a) more rapid development so that, as larvae, ridged animals can take advantage of more ephemeral waters in dry years.

(b) the larvae of the morph may be able to tolerate the warmer water temperatures which are found in warm dry seasons.

Both these possibilities are subject to experimental test under constant temperature conditions.

Further studies on the inheritance and genetic control of the morph patterns are desirable. However, the apparent small size of the lighthouse soak population makes it essential that no animals be removed lest interference affect the nature of the selective processes operating on the population. Additional matings can be made in years when the population size is large enough for the effect of removing animals to be reduced or negligible.

Acknowledgments

The work was done with facilities offered by the Rottnest Biological Research Station while the author was in receipt of a Research Grant from the University of Western Australia. Thanks are also offered to Messrs. A. K. Lee and S. Barker for assistance in the collection of field data and to J. H. Calaby and G. M. Storr who read and criticised the manuscript.

References

Hayne, Don W. (1949).—Two methods for estimating populations from trapping records. *J. Mammal.* 30: 399-411.

Hodgkin, E. P., and Sheard, K. (1959).—Rottnest Island; the Biological Station and recent scientific research. *J. Roy. Soc. W. Aust.* 42: 65-95.

Littlejohn, M. J. (1960).—Call differentiation in a complex of seven species of *Crinia* (Anura Leptodactylidae). *Evolution* 13: 452-468.

Loveridge, A. (1935).—Australian amphibia in the Museum of Comparative Zoology, Cambridge, Massachusetts. *Bull. Mus. Comp. Zool. Harv.* 78: 1-60.

Main, A. R. (1957).—Studies in Australian Amphibia I. The genus *Crinia* from south western Australia and some species from south eastern Australia. *Aust. J. Zool.* 5: 30-55.

Main, A. R., Lee, A. K., and Littlejohn, M. J. (1958).—Evolution of three genera of Australian frogs. *Evolution* 12: 224-233.

Moore, J. A. (1954).—Geographic and genetic isolation in Australian amphibia. *Amer. Nat.* 88: 65-74.

Parker, H. W. (1940).—The Australian frogs of the family Leptodactylidae. *Novit. Zool.* 42: 1-106.

Storr, G. M., Green, J. W., and Churchill, D. M. (1959).—The vegetation of Rottnest Island. *J. Roy. Soc. W. Aust.* 42: 70-71.